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MUTAGENESIS: A POTENTIAL APPROACH FOR IMPROVING TOMATOES' NUTRITIONAL QUALITY

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ABSTRACT

In the world, tomato is regarded as one of the important crop. Due to a number of obstacles, tomato production has not yet realized its full potential. Determining the extent of genetic variability for biochemical properties among genotypes of

Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable crops in the world. It is a member of the Solanaceae family, and because of its short life cycle, straight forward diploid genome, accessibility to effective methods for plant transformation, and available genome sequence, it is a promising candidate for mutant breeding systems. Due to its numerous uses in raw, cooked, and processed foods as well as its

Solanum lycopersicum was the goal of this study. Twenty eight (28) M1 genotypes were assessed. Significant differences between genotypes were revealed by the analysis of variance results. Studies on correlation also revealed a variety of correlations between the researched parameters. Lycopene and beta carotene were negatively correlated (r = -0.468**) to each other. Based on non-hierarchical Euclidian cluster analysis, it was demonstrated that there was significant genotype-to-genotype variation. Cluster III was found to have the highest intra-cluster distances, while cluster VI and VII had the highest inter-cluster distances. The clusters with a higher mean value and a greater inter-cluster distance could be chosen as parents for a breeding program. This type of research can aid in the identification of Solanum lycopersicum genotypes with superior yield and biochemical composition. In conclusion, chemical mutagens can be utilized to increase the quantity and quality of tomatoes and have the potential to improve tomato fruit quality.

Keywords: Breeding; Cluster Analysis; Genetic Advance; Genetic Distance; Genetic Diversity; Inter Cluster Distance.

nutritional benefits, tomatoes have recently been in high demand. The major challenges to maintaining the supply internationally include poor yield cultivars, late maturation, environmental changes, and the rapid growth of the human population. According to Juhi, *et al*. (2019), constant cultivation of nourishing and high-yielding tomato cultivars was necessary given the rapidly changing environmental conditions. The limited genetic variety present in farmed tomatoes presents an additional challenge in selecting for high yielding cultivars with

enhanced fruit quality and tolerance to abiotic and biotic challenges. The recent increase in demand for quality improvement and the widespread adoption of fruit quality standards are two notable effects of globalization on horticulture. The impact of plant nutrition on the quality of tomato fruit has recently been the subject of numerous studies. The pedigree approach of hybridization, followed by selection and backcrossing of desired traits, has been used in the early stages of tomato breeding. Early tomato breeding techniques included the pedigree method of hybridization, selection, and backcrossing of desired traits from one parent into another, which led to the development of tomato varieties that were better in terms of yield quantity and quality. Additionally, by utilizing exotic resources and introducing new, desirable genes into the tomato genepool, tomato improvement has grown. The majority of the research on tomato genetics and breeding that has been published recently uses biotechnology and molecular methods. Tomatoes are a "functional food" since they provide a high yield, colour, and flavour to the diet in addition to being a rich source of antioxidants (Harrold, *et al.,* 2007; Ranieri, *et al*., 2004).

Lycopene, beta carotene, ascorbic acid, phenolics, flavonoids, and vitamin E make up the tomato's antioxidant potential. Because of their significance to human health, tomatoes' antioxidant activities may be regarded as a valuable quality trait (Kaur, *et al.,* 2004). Mutation use in plant breeding is known as "plant mutation breeding." It provides good chances for domesticating fruitful underutilized wild species for agricultural or horticultural uses as well as for enhancing newly introduced crops' ability to adapt to unfavourable settings. Due to its simplicity, technical viability, application to all plant species, and usability at both local and large sizes, mutagenesis and plant mutation

breeding have remained popular for almost a century (Seddiqui, *et al.* 2007).

Mutation and mutation breeding, according to (Adamu and Aliyu, 2007), is a method typically used to investigate the structure and operation of genes, which are responsible for plant growth and development. This facilitates the production of genetic improvement raw materials. Chemical mutagenesis is regarded as a potent and crucial method for enhancing crop plants' productivity and quality traits. They went on to say that while sodium azide has been shown to be a chemical mutagen that induces genetic diversity, alkylating chemicals are particularly powerful mutagens in higher plants. Chemical mutagens have also developed into a crucial tool for enhancing the agronomic characteristics of crop plants. Kozgar, *et al*. (2011) and Mostafa (2011) went on to say that mutation breeding has been proven to be extremely effective in raising the genetic diversity for quantitative features in a variety of crop plants.

A mutation is a sudden heritable change in the DNA of a living cell that is not brought on by the frequent occurrence of genetic segregation or genetic recombination. Physical and chemical mutagens are responsible for altering the genetic materials of an organism (typically DNA) and increasing the number of mutations above the natural background level. DeVries suggested using radiation to cause mutations in 1905. In addition, Adelanwa (2011), highlighted that a mutation is a sudden random change in a cell's genetic make-up that may cause it and its offspring cells to deviate in some way from the typical type.

Materials and Methods

The Ladoke Akintola University of Technology, Ogbomoso postgraduate research laboratory is where the study was carried out.

For this investigation, *Solanum Lycopersicum* (tomato) seeds from 24 different germplasms were employed. Five landraces' seeds were gathered from local farmers in Ogbomoso, and one from Oke Ogun, while the seeds of 18 accessions came from the National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan (18). They are all in Oyo State, Nigeria. The study was carried out at the research plots in the Botanical Garden of the Ladoke Akintola University of Technology, Ogbomoso Department of Pure and Applied Biology.

Among 24 varieties of tomatoes plants evaluated only four (4) varieties were selected for mutation induction. They were two landraces and two accessions:

1. ABE,
2. ALA
3. NHGB 09/120 and 4.NGB 01410

This selection was based on high yield and lycopene content for each one of the variety.

Twenty eight mutants and control of *Solanum lycopersicum* of four accessions selected were used. Six biochemical tests on tomatoes were conducted using M1 accessions, which were developed using the chemical mutagens sodium azide (NaN_3) and ethyl methyl sulphate (EMS) with the concentrations of 40mM, 60 mM and 80mM for EMS while 2.0 mM, 3.0 mM and 4.0 mM were used for sodium azide.

Table 1: Biochemical contents tested in tomatoes fruits

S/N	Code	Biochemical
i	pH	pH of matured fruit
ii	SOLD	Solid content (g) %
iii	CTRIC	Titratable acidity (citric acid in %)
iv	LCP	Lycopene content (µg/g)

v	BETA	Beta-carotene content (µg/g)
vi	RDU	Reducing sugar content (µg/g)

Protocol of determination of the biochemical parameters of the tomatoes

The following explanations were given for the procedures used to determine the biochemical parameters:

i. Determination of pH

Slurry method was used to determine the pH level of each variety. To create slurry, prepared 50grams samples of tomato and 100grams of deionized water / distilled water. Puree the sample with the deionized water until a uniform paste is achieved (i.e. ratio 1: 2). Distilled or deionized water has little impact on pH readings because there are not many hydrogen ions present, which is what pH is measuring. Slurry allows the sample to surround the electrode and read more precisely when evaluating solid or semi-solid materials with a spherical-tip electrode (Kyle and Hildebrant 2016). A digital pH-metre was used to calculate the pH. According to Abdul-Hammed, *et al.* (2013) and Abdul-Hammed, *et al.* (2014) the metre was calibrated with buffer 4 and 7.

ii. Determination of the total solid content

Five grams (5) of ripe mixed tomato serum were measured into weight (w_1) crucibles that were clean and dry. The melting pots / crucibles were placed in an oven set at 105°C for approximately 3 hours to achieve a constant weight for the contents (Abdul-Hammed, *et al.* 2014), after being removed from the oven, the crucibles / melting pots were placed in the desiccators to cool. The new weight was taken as w_2. The percentage (%) total solid contents of the tomatoes were calculated as follows:

$$\% \text{ Total solid } = \frac{W2 - W1}{\text{weight of the sample}} x\ 100$$

iii. Determination of the titratable acidity

By titrating a homogenized tomato sample with 0.01 Molar NaOH and utilizing the phenolphthalein indicator, the titratable acidity (TA), given as % citric acid, was discovered (AOAC, 1999). A clean beaker or conical flask was used to weigh four grams of the blended tomato serum. To dilute the serum, 40 millilitres (ml) of distilled water was then added. The solution was filtered, 20 ml of the filtrate was placed into a different, clean conical flask, 2-3 drops of 1% phenolphthalein indicator were added, and the mixture was then titrated against a 0.01 molar NaOH solution until a pink colour was noticed (Abdul-Hammed, *et al.,* 2009). Then quantity of NaOH solution (the average titre) was determined appropriately as a percentage of citric acid, the titratable acidity was computed as follows:

$$\% \text{ Titratable acidity } = \frac{\text{average titre x } \quad 0.0007005}{\text{weight of the sample}} x\ 100$$

iv. Carotenoid extraction method

The tomatoes were chopped into smaller pieces after being rinsed with distilled water. The entire tomato was blended until homogeneous. The carotenoids (lycopene and beta-carotene) were extracted using the modified solvent extraction method (Abdul-Hammed, *et al.,* 2009). A round bottom flask was used to hold the homogenized sample while another round bottom flask was used to quantify 10 g of the sample. The solvents employed were 200 proof 100 % methanol acids, acetone, and n-hexane. According to Perkins-Veazie, *et al.* (2001), it was blended at a ratio of 2:1:1 (two parts n-hexane, one part acetone, and one part methanol). The solvent was mixed with it and 100 ml of it was put into a separating funnel that has been mounted on a

retort stand. The mixture was then given time to separate into an aqueous layer and an organic layer. The aqueous layer was run off and discarded. Twenty millilitres (20ml) of the alcoholic KOH (doubly distilled water, methanol, and 20 % KOH at a ratio of 1:1:1) was treated with the remaining component in the separating funnel in order to saponify any potential triglycerides (Abdul-Hammed, *et al.,* 2013).

The mixture was stirred once more, allowed to separate into layers, and the aqueous layer was afterwards run off and discarded. The leftover mixture in the separating funnel was then washed with 25 ml of distilled water, and 1ml of the organic extract was pipetted into a test tube using a 1000 litres micropipette. N-hexane was used to dilute the extract by a factor of ten (Kyle and Hildebrant 2016). The extract's absorbance at wavelengths between 400 nm and 600 nm was measured using an ultraviolet/visible spectrophotometer on the diluted sample in comparison to n-hexane. For each of the tomato samples, the procedure was repeated.

Estimation of lycopene and beta-carotene concentrations in the analyzed extracts

According to Abdul-Hammed *et al.*, (2013) and Fish *et al.*, (2012), the concentrations of lycopene and beta-carotene were estimated as followed:

$$\text{(Lycopene), µg} = \frac{\text{A505}}{1.72 \text{ x } 10^5} \; x \; 10^6$$

$$\text{Beta-carotene, µg} = \frac{1.483\, A_{487} - \text{A}_{505}}{1.72 \text{ x } 10^5} \; x \; 10^6$$

$$\text{Concentration (µg/g)} = \frac{C(\mu M) x \; molar \; mass \; x \; volume \; of \; n{-}hexane}{1000}$$

$$\text{Concentration (µg/g)} = \frac{Concenration \; (\mu g)}{Weight \; of \; the \; sample \; (g)}$$

(Abdul-Hammed *et al*, 2013).

v. Determination of reducing sugar contents of tomatoes

A dilution factor of one hundred (100) was achieved by combining one millilitre (1 ml) of the homogenized tomato fruits with 19 ml of distilled water, followed by the addition of 80 ml of distilled water. After adding one millilitre (1ml) of 5 % phenol and five millilitres (5 ml) of concentrated sulphuric acid to one millilitre (1 ml) of the prepared solution in a test tube, the mixture was allowed to cool. Using a UV/Visible spectrophotometer and a blank of concentrated sulphuric acid at 490 nm, the absorbance was measured. According to the calibration that was obtained, the amount of reducing sugars was calculated (Abdul-Hammed, *et al.,* 2013).

Calibration using reducing sugar standard

In order to create a dilution factor of 100, one gram of reducing sugar standard (1 g) was weighed into a conical flask, dissolved in 20 ml of distilled water, and then 80 ml of distilled water was added to it. One millilitre (1 ml) of this solution was added to a test tube along with one millilitre (1 ml) of 5% phenol and five millilitres (ml) of concentrated sulphuric acid. The combination was then allowed to cool at a temperature between 280 and 330 degrees Celsius. Using a UV/Visible spectrophotometer, the absorbance of 1 ml of the combination at dilutions of 5, 10, 20, 30, 40, 50, and 100 was measured at 490 nm. The calibration curve for abstaining samples showed the lowering sugar levels (Abdul-Hammed, *et al.,* 2013).

Results and discussion

Results

The use of EMS and sodium azide for mutant breeding dramatically changed the average values of the yield-contributing characteristics and biochemical contents examined in the M1 generation. All tomato

mutant species and accessions showed variability in the mean values for the same attribute in the M1 generation. Additionally, Table 2 shows that for all the characters examined in the current analysis, variability was verified to be higher in the EMS and sodium azide treatments than the control (untreated population).

For the majority of species, it was discovered that mutagenic treated plants had higher biochemical content metrics than control plants. The greatest values were found at pH 4.68 and solid content 9.89 %, respectively, for the highest concentration of NaN_3 (4.0 mM). The characters pH and Solid content (5.04 and 12.42 %, respectively) had the highest values for EMS 60 mM, whereas the character BET (7.41 µg) had the highest value for 3.0 mM of NaN_3 (Table 2). Table 2 lists the variations and effects of mutagens on the biochemical components of each accession. With the exception of EMS (40mM and 60mM) mutants NHGB09/120, all mutant varieties had less lycopene content in the control than in mutant varieties, whereas control varieties had more beta-carotene. Additionally, it was demonstrated that EMS and NaN_3 outperformed control for the majority traits studied.

Table 2: Duncan Multiple Range Test (DMRT) shows effects of mutagen on biochemical contents of tomatoes (*Solanum Lycopersicum*) at M1

S/N	Varieties	Mutagen concentration	Genotype name	pH	SOLD (%)	CTRIC (%)	LCP (µg/g)	BET (µg/g)	RDU (µg/g)
1	ABE	Control	ABCL1	4.65b	8.28^{de}	0.19^{ghi}	8.91^{fgh}	6.91^{ab}	8.05^{f}
2		EMS 40 mM	ABL2	4.67^{b}	9.08^{cd}	0.18^{hi}	12.63^{abc}	3.65^{fghi}	8.72^{abcd}
3		60 mM	ABL3	5.04^{a}	9.68^{bc}	0.18^{hi}	11.90^{abc}	5.87^{abcde}	8.85^{ab}
4		80 mM	ABL4	5.01^{a}	9.66^{bc}	0.17^{hi}	9.41^{fg}	5.31^{bcde}	8.96^{a}
5		NaN_3 2 mM	ABL5	4.32^{defg}	9.00^{cd}	0.27^{abc}	11.33^{cde}	4.97^{defg}	8.54^{abcde}
6		3 mM	ABL6	4.64^{b}	7.78^{e}	0.24^{bcdef}	11.56^{bcd}	5.50^{bcde}	8.42^{cdef}
7		4 mM	ABL7	4.62^{b}	7.46^{ef}	0.25^{bcde}	11.61^{bcd}	4.63^{efgh}	8.45^{bcdef}
8	ALA	Control	ALCL1	4.90^{h}	9.29^{cd}	0.28^{ab}	10.19^{def}	5.70^{bcde}	8.49^{bcde}

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9		EMS 40 mM	ALL2	4.10^{h}	12.02^{a}	0.27^{abc}	12.41^{abc}	4.63^{fgh}	8.56^{abcde}
10		60 mM	ALL3	4.17^{gh}	12.42^{a}	0.26^{abcd}	11.80^{bcd}	3.46^{ghi}	8.64^{abcde}
11		80 mM	ALL4	4.12^{h}	10.39^{b}	0.30^{a}	13.55^{a}	3.14^{hij}	8.58^{abcde}
12		NaN_3 2 mM	ALL5	4.19^{fgh}	8.33^{de}	0.27^{abc}	13.13^{ab}	5.01^{defg}	8.67^{abcde}
13		3 mM	ALL6	$4..24^{fgh}$	9.40^{bc}	0.28^{abc}	13.0^{abc}	5.96^{abcde}	8.78^{abc}
14		4 mM	ALL7	4.35^{defg}	9.89^{bc}	0.25^{abcd}	12.60^{abc}	5.43^{bcde}	8.74^{abc}
15	NHGB09/120	Control	NHCL1	4.18^{gh}	5.60^{ghi}	0.20^{fghi}	11.70^{bcd}	$4.66e^{fgh}$	5.13^{g}
16		EMS 40 mM	NHL2	4.33^{defg}	6.30^{g}	0.20^{efghi}	12.76^{abc}	2.11^{ij}	3.33^{i}
17		60 mM	NHL3	4.37^{def}	5.50^{ghi}	0.19^{ghi}	12.59^{abc}	2.82^{ij}	4.01^{h}
18		80 mM	NHL4	4.34^{defg}	5.74^{gh}	0.18^{hi}	9.45^{fg}	5.45^{bcde}	3.19^{i}
19		NaN_3 2 mM	NHL5	4.21^{fgh}	5.59^{ghi}	0.21^{efghi}	8.05^{hi}	6.90^{ab}	3.33^{i}
20		3 mM	NHL6	4.25^{efgh}	5.82^{gh}	0.19^{ghi}	5.15^{j}	7.41^{a}	3.01^{i}
21		4 mM	NHL7	4.17^{gh}	6.60^{fg}	0.25^{bcd}	6.91^{i}	6.37^{abcd}	3.49^{i}
22	NGB01410	Control	NGCL1	4.27^{efgh}	4.58^{ij}	0.16^{i}	8.90^{fgh}	5.19^{cdef}	8.27^{ef}
23		EMS 40 mM	NGL2	4.55^{bc}	4.84^{hij}	0.23^{cdefg}	8.00^{ghi}	1.86^{j}	8.51^{bcde}
24		60 mM	NGL3	4.50^{bed}	5.03^{hij}	0.27^{abc}	7.50^{hi}	4.97^{defg}	8.67^{abcde}
25		80 mM	NGL4	4.63^{b}	4.25^{ik}	0.22^{defgh}	11.94^{abc}	5.24^{cdef}	8.69^{abcde}
26		NaN_3 2 mM	NGL5	4.67^{bc}	3.50^{k}	0.23^{bcdefg}	9.79^{ef}	5.86^{bcde}	8.45^{bcdef}
27		3 mM	NGL6	4.43^{cde}	4.22^{ik}	0.22^{defgh}	6.88^{i}	6.77^{abc}	8.56^{abcde}
28		4 mM	NGL7	4.68^{b}	4.36^{ik}	0.20^{ghi}	7.64^{hi}	5.77^{bcde}	8.30^{abcdef}

***Note*:** Mean in a column with any group followed by the same letters are significant difference using Duncan Multiple Range Test (DMRT); **pH = p**H of ripe fruit; **SOLD** =Solid contents (%); **CTRIC =** Citric acid (%); **LCP (µg/g) =** Lycopene content; **BET (µg/g)** = Beta carotene content; **RDU (µg/g)** = Reducing sugar content

The Table three correlation cooefficent studies found a variety of correlations between the characteristics that were looked at. Table 3 lists eight positive correlation coefficients, six of which were significantly associated at $p < 0.01$ (Table 3). Lycopene (LCP) and the solid content of fruit (SOLD), and reducing sugar (RDU) versus the solid content of fruit (SOLD) had the highest positive correlations (r = 0.439** and 0.342**, respectively), followed by citric acid (CTRIC) and

solid content (SOLD) (r = 0.242**) and reducing sugar (RDU) versus lycopene content (LCP) (r = 0.233**, respectively). Additionally, three of the association coefficients (Table 3) were negatively and statistically significant at ($p < 0.05$ and $p < 0.01$). They were beta carotene (BET) versus lycopene (LCP) (r = -0.499**), solid content (SOLD) versus pH of mature fruit (r = -0.143*), and citric acid (CTRIC) versus pH (-0.284**). Additionally, several show negligible negative correlations that are not statistically significant, such as LCP versus pH (-0.084) and RDU versus BET (-0.042).

Table 3: Shows the correlation coefficients of biochemical contents of tomatoes measured at M1 generation

Traits	pH	SOLD	CTRIC	LCP	BET	RDU
pH	1					
SOLD	-0.143*	1				
CTRIC	-0.284**	0.242**	1			
LCP	-0.084	0.439**	0.177**	1		
BET	0.062	-0.104	-0.037	-0.499**	1	
RDU	0.141*	0.342**	0.222**	0.233**	-0.042	1

Note: * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

pH**=** pH of matured fruits; SOLD= Solid content of matured fruits (%); CTRIC= Citric acids in %; LCP= Lycopene in micro gram per gram (µg/g); BET= Beta carotene in microgram per gram (µg/g); RDU= Reducing sugar content in microgram per gram (µg/g)

All six of the qualities investigated in the heritability study have strong heritabilities. The link between environmental impacts (ECV) and genetic effects (GCV) is demonstrated by the induced phenotypic

variability (PCV). For all of the qualities, PCV was greater than GCV (Table 4).

Table 4: Variance components, heritability in broad sense (h^2b), genetic advance (GA) and genetic advance over mean (GAM) for biochemical contents of tomatoes (M1) mutants varieties.

Traits	Range			δ^2e	δ^2g	δ^2p	GVC	PVC	h^2b	GA	GAM
	Min	Mean	Max								
pH	4.00	4.425	5.20	0.0026	0.065	0.0676	5.7616	5.8757	96.1539	0.515	11.6384
SOLD	3.50	7.308	12.42	2.3043	9.156	11.4603	14.1212	15.7985	79.8932	6.7055	31.2932
CITRIC	0.14	0.226	0.56	0.0002	0.001	0.0012	13.9924	15.3279	83.3333	0.0686	30.361
LCP	1.33	10.405	14.76	0.2777	5.157	5.4347	21.8251	22.4050	94.8902	4.6177	44.3792
BETA	0.65	5.055	10.93	0.2489	1.725	1.9739	25.9821	27.7934	87.3905	2.7829	55.0523
RDU	2.78	7.345	9.35	0.0161	4.759	4.7751	29.7007	29.7509	99.6628	4.3284	58.9296

Eight clusters were formed from the twenty-eight (28) genotypes of *Solanum lycopersicum* (Table 5 and Dendrogram of relationship in Figure 1). Cluster-I, which comprises a total of nine (9) genotypes, or 32.14 % of all the genotypes analyzed, created the greatest number of clusters. The Cluster which follow cluster one was cluster VIII which the value of four (4) genotypes. In addition, clusters III, IV, V, and VII followed them, with genotypes of 3 and 10.71 % apiece, but clusters II and VI have the fewest genotypes, with 2 and 1 genotypes, respectively.

Table 5: Clusters of 28 *Solanum lycopersicum* genotypes

Cluster	No. of genotype	Percent (%)	Genotype name
I	9	32.14	ABL6, ABL7, ABL5, ALL6, ALL7, ABL3, ABL2, ALL5, ALL4
II	2	7.14	ALL2 and ALL3
III	3	10.71	ABL4, ALCL1 and ABCL1

IV	3	10.71	NGCL1, NGL5 and NGL4
V	3	10.71	NGL3, NGL7 and NGL6
VI	1	3.57	NGL2
VII	3	10.71	NHL2, NHL3 and NHCL1
VIII	4	14.29	NHL5, NHL7, NHL4 and NHL6

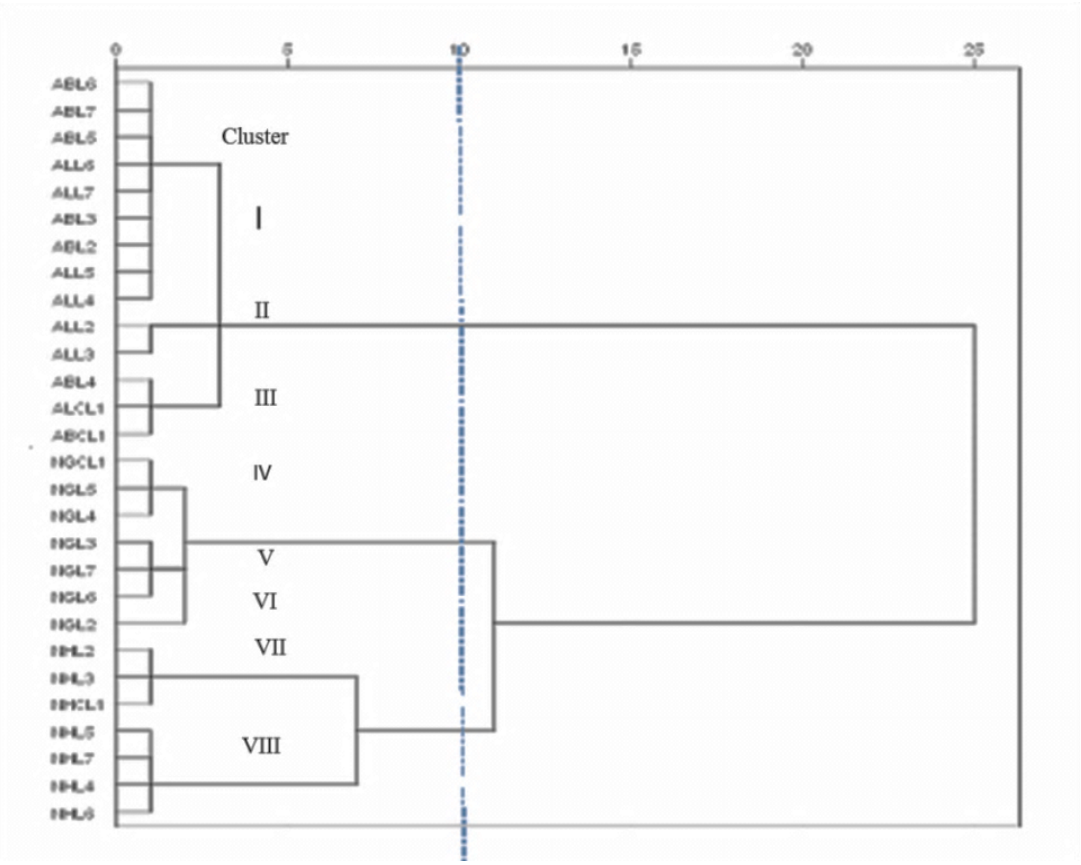

Figure 1: Dendrogram of relationship among twenty eight mutants tomato studied

Table 6: Estimates of inter- and intra – distances for biochemical traits in *Solanum Lycopersicum* accessions and landraces

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**1.186**	6.392	5.066	4.771	3.214	8.141	7.201	4.809
II		**0.636**	6.602	6.821	7.691	7.653	7.426	3.376

III			**1.762**	6.355	5.760	3.350	7.950	5.652
IV				**0.000**	3.837	8.401	7.475	6.034
V					**0.996**	8.792	5.612	5.296
VI						**1.276**	10.212	7.800
VII							**1.309**	4.370
VIII								**1.559**

Table 7: Mean values of six traits of 28 *Solanum lycopersicum* genotypes

Traits	pH	SOLD	CTRIC	LCP	BETA	RDU
Cluster I	4.47	9.00	0.25	12.38	4.91	8.64
Cluster II	3.35	12.22	0.27	12.11	4.05	8.60
Cluster III	4.85	9.08	0.21	9.50	5.97	8.50
Cluster IV	4.52	4.11	0.20	10.21	5.43	8.47
Cluster V	4.55	4.54	0.23	7.34	5.84	8.51
Cluster VI	4.55	4.84	0.23	8.00	1.86	8.51
Cluster VII	4.29	5.80	0.20	12.35	3.20	4.16
Cluster VIII	4.24	5.88	0.21	9.03	5.85	3.79

Distance Analysis

Significant genotypic differences were seen both within and between clusters, as indicated by the intra and inter cluster distance. The intra cluster distances (Table 6) varied from 0.00 to 1.762. Cluster III (D2 = 1.762), cluster VIII (D2 = 1.559), cluster VII (D2 = 1.309), cluster VI (D2 = 1.276), and cluster I (D2 = 1.186) were the clusters with the largest intra cluster distance. Cluster III's maximum intra-cluster distance (D2 = 1.762) demonstrates that the genotypes in this cluster differed more from those in other clusters. Additionally, the largest inter-cluster distances (Table 6) were found between Clusters VI and VII (D2 = 10.212), Clusters V and VI (D2 = 8.792), Clusters IV and VI (D2 = 8.401),

and Clusters I and VI (D2 = 8.141). This suggests that the occurrence of maximum genetic distance between genotypes inside such clusters can result in effective genotypes or progeny that help build varieties that perform better than those that have already been issued.

The shortest inter-cluster distance was found to be between clusters I and V (D2 = 3.214), followed by clusters III and VI (D2 = 3.350), clusters II and VIII, and clusters IV and V (D2 = 3.837). This shows that these clusters are quite distinct from one another and that the genotypes that belong to them can be employed in a hybridization scheme.

Mean Values of the Clusters

The mean performances of Cluster-I, which had nine (9) genotypes, were highlighted by the greatest cluster mean values for lycopene concentration and reducing sugar content. Cluster II had two genotypes and was distinguished by having the largest solid content and the second-highest cluster mean value for the amount of reducing sugars, but it was also distinguished by having the lowest cluster mean value for pH. Three genotypes made up Cluster III, which was thought to have the highest cluster mean value for beta carotene.

With regard to solid content, the fourth cluster (IV) had the lowest cluster mean value. The lowest cluster mean values for lycopene concentration were found in Cluster V, which had three genotypes. Only one genotype made up Cluster VI, which had the lowest cluster mean value for beta-carotene. The second lowest cluster mean values for beta carotene and the second lowest mean cluster value for reducing sugar were found in Cluster VII, whereas the lowest cluster mean values for reducing sugar content were found in Cluster VIII (Table 7).

Discussion

Crop types with crucial unique features, such better growth and production potential, are required to feed the world's population as it

continues to rise. Plant breeding is an old science that was crucial to the green revolution since it helped to boost crop yields. Mutation offers a means of getting around issues with existing plant enhancement methods. The use of mutagens in plant breeding makes it simple to recognize the system of mutation induction and to clearly identify both the rate and pattern of changes caused by mutagens in various selected plants.

Mutagens were initially employed directly or indirectly to create new crop varieties. Additionally, mutagens can be utilized to find new genes, regulate crucial features, and comprehend how genes work. Compared to genetically modified organisms (GMO), mutants are more attractive. An excellent resource for creating new types is a mutant population with a high density of mutations, according to (Meena, *et al.,* 2017).

Information on genetic variables or parameters, such as mean, genotypic variance, phenotypic variance, heritability, and genetic advance for various traits, is used in crop improvement research to indicate if a trait can be improved by selection. In the current investigation, ethyl methyl sulphate and sodium azide (chemical mutagens) were used as mutagenic treatments that resulted in variability in M1 production for several characteristics. However, the effects of this heterogeneity in biochemical characteristics varied with concentration levels. Six biochemical traits' genetic components were analyzed in several cultivars to determine the potential for improvement through selection.

In the current study, ABE had the highest pH and reducing sugar values, ALA had the highest levels of citric acid and lycopene, and NHGB 09/120 had the highest levels of beta-carotene. The percent total solid for all the controls in this study varied from 4.58% to 9.29%. The Ogbomoso landrace (ALA) had the highest value, 9.29%, while

NGB01410 received the lowest value, 4.58%. It varies between 3.50% NGB01410 2.0 mM NaN_3 and 12.42% ALA 60 mM EMS for the mutants. This finding demonstrates that mutants outperformed controls. According to this finding, which is comparable to that of (Abdul-Hammed, *et al.,* 2009), tomatoes constitute 93–95 percent water, with other ingredients including inorganic and organic chemicals.

Variations were seen with the different tomato varieties as well as differences in the lycopene levels, ripening, and antioxidant indices of the various tomato mutants. The average lycopene content of fresh tomatoes were measured at 13.550 micrograms per gram (µg/g). Lycopene, a carotenoid that makes up between 75% and 83% of all carotenoids, gives fruit its red colour. The main source of lycopene is the tomato, which has high levels but varies depending on the harvest season, the region, and the genotype of the plant.

Perhaps the most well-known carotenoid is beta-carotene. With no oxygen atoms in its chemical structure, beta-carotene is a real carotene, as its name implies. A naturally occurring pigment with a reddish-orange hue, beta-carotene is most frequently found in fruits and vegetables. A pro-vitamin for vitamin A is beta-carotene. Pro-vitamins are forms of a vitamin from which the body can produce another vitamin. Because they are nutrients, carotenoids are also essential to humans (Abdul-Hammed, *et al.,* 2014) and the amount of beta-carotene varies greatly between species or tomatoes mutations. Lycopene and beta-carotene had a negative correlation, with the beta-carotene content decreasing as the lycopene content increased. Therefore, the researchers can cross breed ALA with maximum lycopene content with that of NHGB 09/120 with greatest beta carotene content in order to improve lycopene and beta carotene contents.

It was proven that most M1 families exposed to mutagens displayed higher variability when compared to controls. The link between environmental impacts (ECV) and genetic effects (GCV) is demonstrated by the induced phenotypic variability (PCV). Additionally, for all six biochemical traits, most mutagenic treatments caused broader ranges of variance in treated families than in controls, and the amount of this variation in features was either increased or decreased. In a related study, Meena, *et al.* (2017) recorded detail differences between GCV and PCV estimates for grain yield and its component in Field pea (Pisum sativum).

The clustering pattern in this study demonstrated that tomatoes from various geographic regions were clustered together in a cluster, and vice versa demonstrated that geographic diversity did not always imply genetic diversity. This was consistent with findings from Meena, *et al.* (2022) as earlier D2 analysis. The fact that the tomatoes came from the same place but were organized into separate clusters suggested that they might have undergone changes due to effects of mutagens used.

Cluster III's highest intra-cluster distance (1.762) can be used as a reference for choosing parents for in-cluster hybridization. Additionally, clusters VI and VII had the greatest inter-cluster distances (10.212), followed by clusters V and VI (8.792), which showed that the mutants in these groups had a great deal of genetic diversity. Choosing parents from these clusters for hybridization programs would help to produce the desired results. The shortest inter-cluster distance revealed a close link between the mutations in these clusters. Therefore, choosing parents from these two clusters should be avoided. On 49 genotypes of kabuli chickpea investigated by Temesgen, *et al.* (2015), genetic variation was grouped into eight clusters. A similar outcome was obtained by Gizachew and Gebeyaw

(2021), which grouped 49 genotypes of chickpea (*Cicer arietinum L.*) into eight clusters and discovered that there was a significant genetic gap across genotypes.

In plant breeding, clusters with a greater inter-cluster distance and a higher mean value will typically result in divergent trees. Therefore, high inter-cluster distance clusters VI and VII could be chosen as parents for breeding programs. This type of research can aid in the identification of *S. lycopersicum* genotypes with superior biochemical composition.

Conclusion and recommendation

In conclusion, tomato (*S. lycopersicum*) research has advanced significantly in all areas, making it one of the most studied horticulture crops. The use of mutagens in crop improvement has aided in the understanding of the technique of inducing mutations as well as the estimation of the frequency and pattern of changes caused by mutagens in various chosen plants. These mutagens' capacity to infiltrate living organisms' cells and interact with DNA results in the general harmful effects linked to their mutagenic capabilities. Therefore, the primary cause of their effects is the direct interaction of the mutagen with DNA molecules. In addition to producing different types of resistance, sodium azide and EMS mutagenesis offer an effective technique for breeding disease-resistant cultivars. To increase the nutritional value of tomato fruits, chemical mutagens were applied. It was clear that developed mutagens approaches have the ability to improve fruit quality to satisfy 21st-century needs. The authors recommended that using chemical mutagens such as EMS and sodium azide will improve quality and quatity of tomatoes fruits.

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